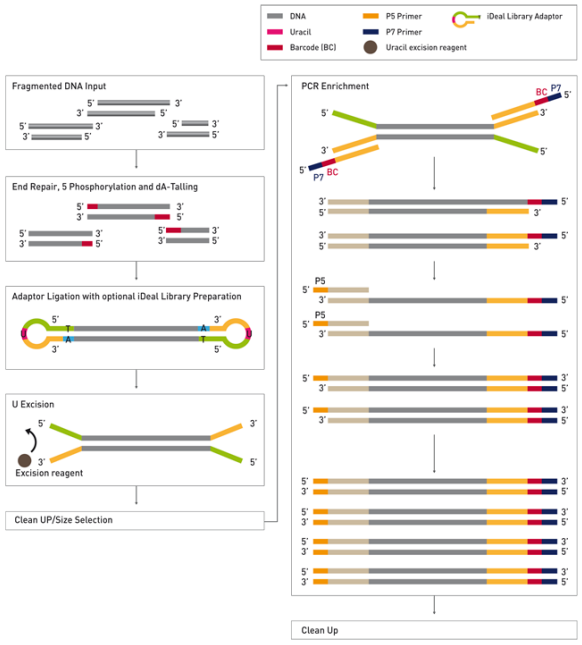
IP-Star® Compact

Ideal Library Preparation kit

1. About the protocol

The "**Ideal_Library_Preparation**" protocol on the IP-Star® is using the standard "**Ideal Library Preparation kit**" and reagents from **Diagenode**. The **Ideal Library Preparation kit** allows the preparation of indexed libraries of **genomic** or **ChIP DNA**.
It provides flexibility to prepare 1 to 32 libraries in one run starting with **5ng of DNA**. The whole protocol takes approximately 1h30. It allows you to prepare up to 96 libraries per day with 3 runs. At the end, you recover ligated products ready for amplification.

2. Workflow



3. Material required

a. Reagents & kits

Item	Supplier	Catalogue #
iDeal Library Preparation Kit x24 (incl. Index Primer Set 1)	Diagenode	C05010020
iDeal Library Index Primer Set 2 (optional)	Diagenode	C05010021
Agencourt® AMPure® XP Beads	Beckman Coulter®	
Fresh Ethanol 80%	Lab supplier	

b. Cconsumables

Item	Supplier	Catalogue #
200 µl tube strips (8 tubes/strip) + cap strips	Diagenode	C30020002
Tips (box)	Diagenode	C30040021
Tips (bulk)	Diagenode	C30040020

4. IP-Star setup

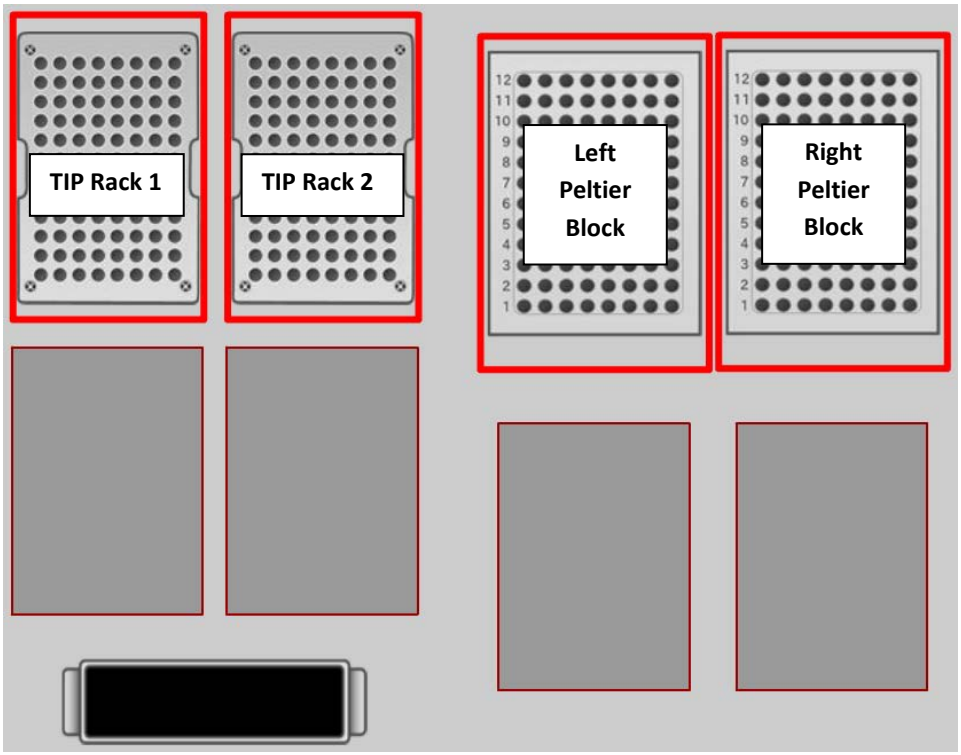
1. Switch ON the IP-Star.
2. Select "**Protocols**" icon and then click on "**Library prep**".
3. Under "**Library prep**", select "**Ideal_Library_Preparation**".

Note:
If you plan to run between 1 and 8 samples, chose "**Ideal_Library_Preparation_08**"
If you plan to run between 9 and 16 samples, chose "**Ideal_Library_Preparation_16**"
If you plan to run between 17 and 24 samples, chose "**Ideal_Library_Preparation_24**"
If you plan to run between 25 and 32 samples, chose "**Ideal_Library_Preparation_32**"

4. Setup the exact number of samples that you want to process.

Note:
The **Left Peltier Block** is now cooling down to 4°C to keep the enzymes and reagents cold.

5. Setup all the plastics on the platform according to the screen layout.



6. Fill **TIP Rack 1** (and 2 if processing more than 8 samples) with tips according to the screen.
7. Fill **Left and Right Peltier Blocks** with 200 µl tube strips according to the screen.

5. Reagents & Samples setup

Note:
Allow the reagent from "**Ideal Library Preparation kit**" to come at 4°C.
Work on ice from this point.

8. Prepare the following mixes.

   - **Ideal Library End Prep Mix:**

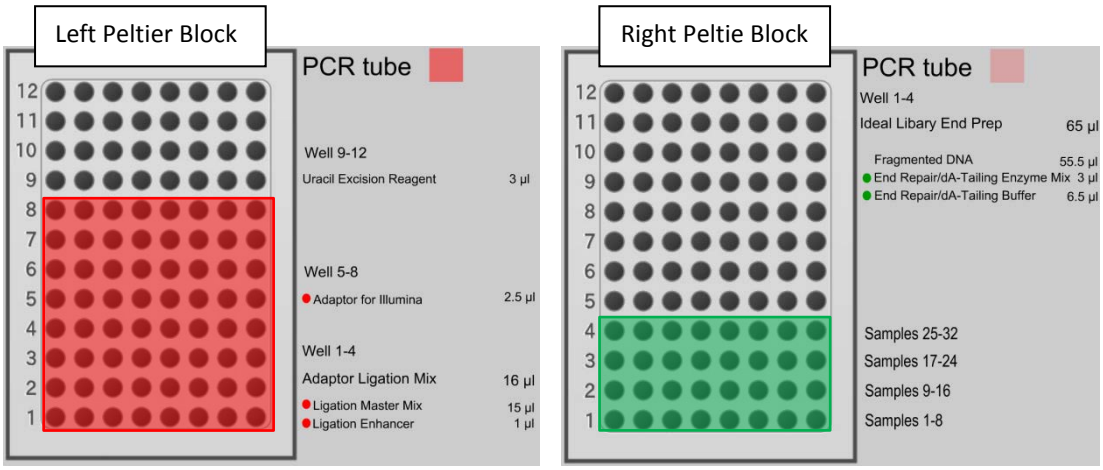
	# 1	# 8	# 16	# 24	# 32
iDeal Library End Repair/ dA-Tailing Enzyme Mix (green)	3 µl	24 µl	48 µl	48 µl	72 µl
iDeal Library End Repair/ dA-Tailing Buffer (green)	6.5 µl	52 µl	104 µl	156 µl	208 µl
TOTAL	**9.5 µl**	**76 µl**	**152 µl**	**204 µl**	**280 µl**

Note:
55.5 µl of DNA will be added later for each sample.

   - **Ideal Library Adaptor Ligation Mix:**

	# 1	# 8	# 16	# 24	# 32
iDeal Library Ligation Master Mix (red)	15 µl	120 µl	240 µl	360µl	480 µl
iDeal Library Ligation Enhancer (red)	1 µl	8 µl	16 µl	24 µl	32 µl
TOTAL	**16 µl**	**128 µl**	**256 µl**	**384 µl**	**512 µl**

9. Fill the **Left Peltier Block** with **Ligation Mix** according to the screen layout.
10. Fill the **Left Peltier Block** with **2.5 µl iDeal Library Adaptor for Illumina (red)** according to the screen layout.
11. Fill the **Left Peltier Block** with **3 µl iDeal Library Uracil Excision Reagent (red)** according to the screen layout.
12. Fill the **Right Peltier Block** with the **9.5 µl End Prep Mix** according to the screen layout.
13. Add **55.5 µl of DNA** in each sample according to the screen layout.



14. Close the door and Run.

6. Size Selection of Adaptor-Ligated DNA

Please refer to the **Ideal Library Preparation kit** manual on page 7

7. Alternatively, Cleanup of Adaptor-ligated DNA without Size Selection

Please refer to the **Ideal Library Preparation kit** manual on page 8

8. Library Amplification

15. Mix the following components in sterile strip tubes

Adaptor Ligated DNA Fragments	23 µl
iDeal Library PCR Master Mix (blue)	25 µl
iDeal Library Index Primer* (blue)	1 µl
iDeal Universal PCR Primer* (blue)	1 µl
Total volume	**50 µl**

* These primers (Index Primer Set 1) are included. Index Primer Set 2 may be purchased separately (Cat. No. C05010021).

16. Transfer tubes to a pre-programmed thermal cycler and incubate as follows.

Cycle Step	Temp	Time	Cycles
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	6 - 15*
Annealing	65°C	30 seconds	
Extension	72°C	30 seconds	
Final Extension	72°C	5 minutes	1
Hold	4°C	∞	

* Suggestion: 6 PCR cycles for 1 µg DNA input 10 cycles for 50 ng, and 13-15 for 5 ng DNA input. Further optimization of PCR cycle number may be required